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EFFECTS OF PERIPARTURIENT INJECTION OF VITAMIN B_2 ON THE NEUTROPHIL FUNCTIONS AND POSTPARTURIENT REPRODUCTIVE PERFORMANCE IN PREGNANT DAIRY COWS

(With 5 Tables)

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تأثير حقن فيتامين ب٢ فى فترة ما قبل الولادة على وظائف النتروفيل والكفاءة التناسلية للأبقار الحلابة العشار فى فترة ما بعد الولادة

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تم تقييم تأثير حقن فيتامين ب٢ فى فترة ما قبل الولادة على وظائف النتروفيل والكفاءة التناسلية للأبقار الحلابة العشار فى فترة ما بعد الولادة. قد لوحظ زيادة معنوية فى كل من نسبة ومعامل القوة الالتهامية لخلايا النتروفيل فى الأبقار المحقونة بفيتامين ب٢ بعد ٥ أيام من الحقن وأثناء الولادة عن قبل الحقن وعن المجموعة الضابطة. وكذلك لوحظ تحسن واضح فى كل قياسات تقييم الكفاءة التناسلية بعد الولادة. حيث ظهر نقص معنوى واضح فى كل من الوقت اللازم لنزول الأغشية الجنينية وكذلك فى الفترة اللازمة للارتجاع الكامل للرحم وحدوث أول شبق ظاهرى. كما زاد معدل الإخصاب ونقص عدد الوثبات اللازمة لكل إخصاب. وقد ظهر نقص معنوى واضح فى عدد الأيام من الولادة حتى أول حمل بعد الولادة مقارنة بالمجموعة الضابطة.

SUMMARY

Effects of the periparturient injection of vitamin B_2 (VB_2) on the peripheral blood neutrophil functions and postpartum reproductive performance (PRP) in pregnant dairy cattle were investigated. A significant increase in both phagocytic percentage (P%) and phagocytic index (PI) was observed in VB_2 treated cows 5 days after treatment and at parturition than before treatment as well as than those of control cows. Moreover, the peripartum i.v. injection of VB_2 gave an improvement in all the measured PRP. Significant variations noticed between the placental expulsion period, days from calving to uterine involution, days

to the first postpartum heat, conception rate, number of services per conception and open days in comparison with the control group.

Key words: *Cattle, neutrophil, vitamin B_2, postpartum reproductive performance.*

INTODUCTION

The economy of dairy cows depends to a great extent on the reproductive efficiency and milk production, which are the main desired goals in dairy cow breeding.

During the periparturient period, animals undergo marked physiological changes that might cause suppression to the host defense mechanism and an increase in susceptibility to uterine and mammary gland infection (Nagahata *et al.*, 1991). It is proved that, stress of pregnancy, parturition and lactation have pronounced effect on the immune response of the dam (Lioyd, 1983).

Non-specific immunostimulants have been received considerable attention in the veterinary field. They appear to provide an efficient method of stimulating the immune system in a non-specific manner with few adverse side effects (Krakowski *et al.*, 1999). Immunostimulants compounds have the potential to counteract the effect of environmental or microbial immunosuppressive factors. They may thus reduce morbidity and economic losses resulting from subclinical or mild infectious disease (Mayer, 1981).

Therefore, the regulation and potentiation of the immune system have become the subject of high interest in recent years. There is adverse range of substances which are widely used as immunostimulants for enhancing the immune defense mechanism and contract the effect of environment, microbial and stress immunosuppressive factors (Koller, 1982).

Vitamin B_2 (VB_2) has been found to enhance non-specific host defense mechanisms against bacterial infections in mice (Araki *et al.*, 1995 and Kimura *et al.*, 1996) and cows (Osame *et al.*, 1995 and Sato *et al.*, 1999). The present investigation aimed to evaluate the efficacy of VB_2 as reported immunopotentiator in improving the postpartum reproductive performance in dairy cows.

MATERIAL and METHODS

Fifty Friesian cows in the last trimester of pregnancy belonging to a private farm at El-Manzala, Dakahlia, Egypt were used. The animals were allocated to two treatment groups, with 25 cows in each one. The investigated cows were housed in open yards, handled identically and kept under similar managemental practice. Their ages ranged between 4 to 6 years. Each animal was received 3 kg concentrates three times daily in addition to 25kg good quality seasonal green fodder. These animals had been regularly tested for freedom of genital diseases. The prophylactic immunization system for the animals included annual vaccination against Foot and Mouth Diseases, Rift Valley Fever and Lumpy Skin Disease. The last vaccination in the studied cows was given 4 months before the beginning of this study. Additionally, all cows were received routine treatment with antihemelmintics.

Preparation of VB_2: VB_2 (Riboflavin sodium phosphate, Wako Pure, chemical Co., Tokyo, Japan) was prepared as a 5% w/v solution in injecTables sterile water (Egyptian International Pharmaceutical Industries Co., A. R. E.).

Treatment protocol: Cows in group I were received single intravenous (i.v.) injection of VB_2 (5 mg/kg). Those in groups II were injected i.v. with the same volume of vehicle (sterile water for injection) alone and served as a control group. The VB_2 and vehicle were injected 30 days prior to the anticipated date of calving.

Blood sampling: Three heprinized blood samples were collected from each animal via Juglur vein. Puncture the first sample was taken before treatment, the second was taken 5 days after treatment and the third one was taken at parturition

Immunological studies: Peripheral blood leukocytes were counted with a heamocytometer and the neutrophil counts were estimated from the differential count determined using Giemsa-stained blood smears (Schalm *et al.*, 1975). Isolation and phagocytic activity of neutrophils were performed by the method described previously (Lehrer and Clins1989). Briefly, Hank's Balanced Salt Solution (Sigma Chemical Co., U. S. A.), neutrophil suspension, previously prepared pooled serum as a source of opsonin and heated killed *Candida*, 0.25ml from each, were mixed and incubated at 37°C for 30 minutes and then centrifugated at 200 r.p.m. for 5 minutes, after which smears were prepared from the sediment, dried in the air and stained with Leishman's stain. The phagocytic percentage was determined by the number of

neutrophils engulf Candida (one or more) among 100 counted neutrophils (Lehrer and Clins1989), while the phagocytic index was determined by the number of Candida engulfed by 100 neutrophils divided by 100 (Vandeplassche and Bouters, 1983).

Assessment of postpartum reproductive performance (PRP): All the investigated cows were kept under strict observation during parturition and were closely observed for dropping of the placenta. The ovaries and the uterus were palpated per rectum twice a week till day 21 and later on once a week till day 42 postpartum to evaluate the uterine involution and ovarian activity. Later on cows were closely observed daily for signs of estrus. Estrus was confirmed by rectal palpation and cows in heat were inseminated using thawed frozen semen according to a.m., p.m. rules. The pregnancy was diagnosed by rectal palpation 42 days post insemination. The obtained data were derived for the mean values of the fetal membranes expulsion period, days from calving to uterine involution, days to first estrus, conception rate, number of services per conception and the open days.

Statistical analysis: Data were expressed as the mean ± stander error. The statistical analysis of observed differences was performed by Student's t- test, and probabilities value (P) less than 0.01 was considered to be statistically significant.

RESULTS and DISCUSSION

Stresses of pregnancy and parturition cause suppression of the host defense mechanism, which is manifested by impairing neutrophil functions and increase susceptibility to uterine infection (Kehrli *et al.*, 1989). Thus improved immune status of late pregnant cows is an important measure to overcome and decrease the losses resulting from reproductive disorders (Lioyd, 1983). Recently, VB_2 was found to enhance non-specific host defense mechanism against bacterial infection (Osame et at., 1995). Therefor, the effect of intravenous injection of VB_2 on neutrophil functions and its efficacy on the postpartum reproductive performance were investigated in the present study.

Effects of VB_2 on the peripheral blood neutrophils: The peripheral blood neutrophil counts in the group I, receiving a single dose of VB_2, displayed slight changes 5-days after administration as well as at parturition and showed non-significant increase from the corresponding values of the control group (Table 1). Moreover, the leukocyte count displayed a similar pattern to the changes in the neutrophil count (Table 2).

Table 1: Effect of periparturient single intravenous injection of vitamin B_2 on the neutrophil count. (Mean ± S.D. X 10^3)

	Total neutrophil count X 10^3	
	Group I (N=25)	Group II (N=25)
Before treatment	2.086 ± 0.82	2.088 ± 0.97
5days after treatment	2.121 ± 0.85	2.084 ± 0.85
At parturition	2.101 ± 0.69	2.078 ± 0.64

N = total number of the cows.

Table 2: Effect of periparturient single intravenous injection of vitamin B2 on the total leukocytic counts. (Mean ± S. D. X 103)

	Total leukocytic counts X103	
	Group I (N=25)	Group II (N=25)
Before treatment	7.562 ± 1.75	7.561 ± 1.25
5days after treatment	7.564 ± 2.61	7.562 ± 1.89
At parturition	7.562 ± 1.95	7.559 ± 2.07

N = total number of the cows.

As regards the phagocytic activity (Tables 3&4), the obtained data revealed that, the control group had lowered phagocytic percentage (P%) and phagocytic index (PI) before and during parturition. The decreased P% and PI in the control group attributed to the stress of pregnancy and parturition (Cai *et al.*, 1994), which causes increase in cortizol level that suppress the phagocytic activity of neutrophil (Whitmore *et al.*, 1988). On the other hand, a significant increase ($P< 0.05$) in both P% and PI was observed in VB_2 treated cows 5 days after treatment and at parturition than before treatment as well as than those of the control cows. Thus, the present study revealed that the intravenous injection of VB_2 to the pregnant cows at the late stage of pregnancy enhance the neutrophil function resulting in activation of the host defense mechanism against bacterial infection. Previous studies have revealed that VB_2 was found to enhance non-specific host defense mechanisms against a variety of bacterial infections in cattle by stimulating the generation of neutrophils and also enhancing neutrophil functions (Osame *et al.*, 1995 and Sato *et al.*, 1999). The mechanism of activation of neutrophil function following VB_2 injection has not been clear. However, it was suggested that the neutrophil function is activated

because of increased and/or regulated cytokinese production induced by VB_2 injection (Sato *et al.*, 1999).

Table 3: Effect of periparturient single intravenous injection of vitamin B2 on the neutrophil phagocytic percentage. (Mean ± S. D.)

	Neutrophil phagocytic percentage	
	Group I (N=25)	Group II (N=25)
Before treatment	64.27 ± 7.28	64.43 ± 8.25
5days after treatment	87.14 ±9.14**	62.30 ± 4.65
At parturition	85.57 ± 5.97**	62.11 ± 5.29

N = total number of the cows. **P< 0.01 vs. control.

Table 4: Effect of periparturient single intravenous injection of vitamin B2 on the neutrophil phagocytic index. (Mean ± S. D.)

	Neutrophil phagocytic index	
	Group I (N=25)	Group II (N=25)
Before treatment	1.63 ± 0.11	1.63 ± 0.14
5days after treatment	5.13 ± 0.71**	1.62 ± 0.12
At parturition	4.95 ± 1.25**	1.60 ± 0.16

N = total number of the cows. **P< 0.01 vs. control.

Effects of VB_2 on the PRP: Although the effect of VB_2 on the non-specific host defense mechanism has been demonstrated, but no reports have appeared concerning the effects of VB_2 on the PRP. The present study revealed for the first time that the peripartum i.v. injection of VB_2 gave an improvement in all the measured reproductive parameters. Significant variations noticed between the placental expulsion period, days from calving to involution, days to first postpartum heat, conception rate, number of services per conception and open days in comparison with the control group (Table 5). The mechanism by which VB_2 improve the PRP is not clear, but it has been reported to increase non-specific resistance against multiple unrelated pathogens like bacteria (Araki *et al.*, 1995). It is possible that VB_2 may have increased the uterine resistance to infection by enhancing cell-mediated immunity against bacteria which might otherwise have reduced the conception rate and increase the service period. Previous studies reported that decreased phagocytic activity of uterine neutrophil in late stage of pregnancy had a detrimental effect on PRP (Quareshi *et al.*,

1997). Further studies will be required to clarify the mechanism by which VB_2 enhanced the uterine defense and improved the postpartum reproductive performance. In conclusion, periparturient i.v. injection of VB_2 to dairy cattle at late stage of pregnancy activated peripheral blood neutrophil and improved the postpartum reproductive performances, so it is beneficial and economic importance to administer such drug in late gestational period.

Table 5: Effect of VB2 on various parameters of the postpartum reproductive performance of cows treated with.

	Group I (N=25)	Group II (N=25)
Placental expulsion period (hours)	8.73± 1.09**	10.96± 1.64
Days from calving to uterine involution	28.61± 0.43**	43.33± 2.19
First estrus interval (days)	38.80± 1.77**	46.11± 1.85
Conception rate (%)	84.00 (n=21)**	64.00 (n = 16)
Number of services per conception	1.41± 0.05**	2.32± 0.51
Open days	85.38± 6.21**	107.58 ± 6.14

N = total number of the cows & n = number of pregnant cows.

Data are expressed as (mean ±S. D.).

*Remarks (**P< 0.01) show significant difference against control group.*

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